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THE EFFECT OF ENVIRONMENT UPON THE PRODUCTION OF SPORANGIA AND SPORANGIOLA IN BLAKESLEA TRISPORA THAXTER[1]

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The purpose of the following experiments was to study the effects of various environmental conditions upon the production of the two types of sporangia in *Blakeslea trispora;* the large solitary sporangia which possess a columella; and the smaller ones, termed sporangiola, which lack a columella and occur in considerable numbers over the surface of large spherical sporangioliferous heads.

The effect of environment upon the production of sporangia in the Mucorales has already been studied by several botanists. Klebs ('96), working with *Mucor racemosus* which has no sporangiola, studied the effect of quantity and quality of the substrate upon sporangial production, also the effects of humidity, atmospheric pressure, temperature, and light. He discovered that the quantity and quality of the substrate play the dominant rôle in the production of sporangia. Tavel ('86) and Bachmann ('95) reported the effect of nourishment upon the formation of the two types of sporangia in *Thamnidium elegans.* Under favorable conditions of nourishment continued through several generations, the sporangiola became as large and contained as many spores as the sporangia. Conversely,

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with poor nourishment, the terminal sporangia changed into sporangiola, often with but one spore. Brefeld ('91) found that under unfavorable conditions of nourishment the terminal sporangia of *Chaetostylum Fresenii* abort, but that they are again produced under favorable conditions. In *Choanephora,* a genus closely related to *Blakeslea,* little experimental work has been reported, although Cunningham ('79), Couch ('29), Möller ('01), and Thaxter ('14) mention that sporangia are generally produced upon exhausted media.

In *Blakeslea trispora,* first described by Thaxter ('14) and studied in the following experiments, conidia are absent and the sporangia and sporangiola show numerous variations and intergradations. The sporangia vary in diameter from 10 to 80 μ (pl. 25, figs. 7 and 10). The sporangiola are sometimes borne upon solitary heads at the ends of erect, unbranched sporangiophores, but usually the heads are in groups of 10 or more (pl. 25, figs. 1–5). The sporangiola are typically 3-spored, rarely 4- or 6-spored, and are attached to the heads by a small spherical vesicle. When mature the sporangiolum falls away, carrying the vesicle with it. The spores are variable in size but in general are alike in all types of sporangia. They are longitudinally striate, and are provided at each end with a cluster of delicate, radiating appendages like those of the sporangiospores of *Choanephora.*

Thaxter ('14) first isolated *Blakeslea trispora* from a culture of *Botrytis* which in turn had been obtained from flowers of the cow-pea. It seems to occur as a weak parasite on various plants (Jochems, '27). It has been shown by Weber and Wolf ('27) to be heterothallic. It is closely related to *Choanephora.* Thaxter homologized the conidia of *Choanephora* with the sporangiola of *Blakeslea,* and suggested that the conidia are to be regarded as monosporous sporangiola. Although he was unable to separate mechanically an outer thin sporangial wall from the conidia of *Choanephora,* his figures of the somewhat abnormal conidia of *C. Cucurbitarum* indicate its existence. In any case, the homology of conidia and sporangia is evident in these genera.

All of the following experiments were performed with two strains of *Blakeslea trispora.* One strain, hereafter called "Strain A," was received through the kindness of Dr. A. F. Blakeslee of the Carnegie Institution of Washington. The other strain, "Strain X," was received from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands. Neither strain deviated greatly from Thaxter's description except that sporangia of the type shown by Thaxter (see pl. 25, fig. 11) were never observed. Both strains produced zygospores when crossed with a minus-strain. The strains differed from each other in several respects. Strain X always produced much more mycelium than did Strain A. In Strain X there were always more sporangia than sporangiola, while in Strain A there were always more sporangiola. Strain A produced more yellow-orange pigment upon potato-dextrose agar. There was no discernible difference, however, in the type and size of the spores, sporangia, or sporangiola of the two strains. While the differences do not seem sufficient to warrant a new species, the two strains could always be easily distinguished when grown upon the same petri dish, and their growth reactions often suggested different species.

EFFECT OF VARIOUS MEDIA

Both strains were used in all experiments. The various media were made up with distilled water. The petri dish cultures were kept in an incubator which varied daily about one degree from a mean of 23° C. unless otherwise stated. All media and utensils were sterilized at 15 pounds pressure for 30 minutes in the autoclave (to be rid of a species of *Bacillus* which invaded the laboratory during the course of these experiments). Single-spore cultures were started from both strains, using the dilution method outlined by Barnes ('35). Spore suspensions were prepared by transferring 10 loopfuls of sporangiola to 15 cc. of sterile water, and 5 drops of this suspension were used in making inoculations. The estimates of the per cent sporangia or sporangiola are not as exact or as quantitative as our tables indicate. They were made from

counts in three or four sectors of petri-dish cultures, using a binocular dissecting microscope. Two petri dishes were used for each strain. The whole experiment was performed twice, and the results were taken from the two experiments (table I).

The relative numbers of sporangia and sporangiola were the same whether spores from sporangia or sporangiola, or even mycelium, were used as inoculum. In the case of potato-dextrose agar, mycelium five months old, dried, and shriveled, was used, but the culture showed the usual distribution of 65 per cent sporangiola with Strain A. The type of sporangia produced was also unaffected by the number of spores used in inoculation, a colony started with one spore having the same distribution as one with from 50 to 100 spores. Repeated transfers had no effect. A colony started from a dish which was the last of a series of 50 transfers had the same relative number of sporangia and sporangiola as that produced by a direct inoculation of spores from the original culture which had not been transferred. No difference was noted in transfers made by platinum needle or spore suspensions in water, nor did the keeping of spore suspensions in distilled water for three weeks affect the results obtained.

Both strains grew most readily upon potato-dextrose agar and the potato agar. In 48 hours the substrate was covered with mycelia and there was abundant fructification. As is seen from the table, in no case was one type of sporangia produced to the exclusion of the other. Upon all media Strain X produced much more mycelial growth than Strain A. Upon Endo's agar and peptone agar Strain A repeatedly produced a preponderance of sporangiola. Mycelial growth was never dense upon Endo's agar and growth was generally restricted. Although sporangiola as well as sporangia were produced upon this media, the distribution in Strain X was quite different from that in the Strain A.

A potato medium was prepared as follows: 5 cc. potato decoction,[1] 95 cc. distilled water, 2 g. agar, and 2.5 g. sugar. The following sugars were used: dextrose, mannose, galactose,

[1] Prepared by steaming 200 gms. of sliced potato in 1000 cc. of water for one hour. The extract was then decanted, filtered, and made up to one litre.

TABLE I

EFFECT OF VARIOUS MEDIA UPON TYPE OF SPORANGIA

Medium	Time of appearance of mycelial growth		Time of appearance of fructification		Distribution of sporangia and sporangiola	
	Strain A	Strain X	Strain A	Strain X	Strain A	Strain X
Rat-dung agar	24 hrs.	24 hrs.	No fruiting	No fruiting		
Yeast-dextrose agar	24 hrs.	24 hrs.	No fruiting	No fruiting		
Nutrient agar	24 hrs.	24 hrs.	No fruiting	No fruiting		
Nutritive caseinate agar	24 hrs.	24 hrs.	No fruiting	No fruiting		
Brain-veal agar	24 hrs.	24 hrs.	No fruiting	No fruiting		
Corn-meal agar	24 hrs.	24 hrs.	72 hrs.	72 hrs.	Sporangia 30% Sporangiola 70%	Sporangia 70% Sporangiola 30%
Eosine methylene-blue agar	24 hrs.	24 hrs.	72 hrs.	72 hrs.	Sporangia 30% Sporangiola 70%	Sporangia 70% Sporangiola 30%
Bacto malt-extract agar	24 hrs.	24 hrs.	48 hrs.	72 hrs.	Sporangia 30% Sporangiola 70%	Sporangia 70% Sporangiola 30%
Banana agar	24 hrs.	24 hrs.	72 hrs.	72 hrs.	Sporangia 40% Sporangiola 60%	Sporangia 70% Sporangiola 30%
Endo's agar	24 hrs.	24 hrs.	72 hrs.	48 hrs.	Sporangia 10% Sporangiola 90%	Sporangia 40% Sporangiola 60%
Potato slices	24 hrs.	24 hrs.	No fruiting	No fruiting		
Peptone agar	24 hrs.	24 hrs.	72 hrs.	72 hrs.	Sporangia 10% Sporangiola 90%	Sporangia 60% Sporangiola 40%
Potato agar	24 hrs.	24 hrs.	48 hrs.	48 hrs.	Sporangia 30% Sporangiola 70%	Sporangia 90% Sporangiola 10%
Urea agar	No growth	No growth				
Ammonium-carbonate agar	No growth	No growth				
Starch-dextrose agar	24 hrs.	24 hrs.	72 hrs.	72 hrs.	Sporangia 15% Sporangiola 85%	No growth
Potato-dextrose agar	24 hrs.	24 hrs.	48 hrs.	48 hrs.	Sporangia 35% Sporangiola 65%	Sporangia 85% Sporangiola 15%

maltose, sucrose, lactose, raffinose, rhamnose, arabinose, xylose, and invert sugar. The distribution of sporangial types was not at all affected in Strain A. Upon all the sugars except lactose, raffinose, and mannose, the mycelium was pigmented, while no pigment was produced upon the control (potato agar without sugar). In Strain X there was much more mycelium but no pigmentation. The control with this strain showed all sporangia and no sporangiola. Mannose gave the same results as the control. All the other sugars showed 85 per cent sporangia to 15 per cent sporangiola.

EFFECT OF QUANTITY OF FOOD

The two strains reacted differently when grown upon agar with decreasing concentrations of potato. Varying amounts of potato decoction, made as previously, were made up to 100 cc. with distilled water, and 2 per cent agar added. Two petri dishes were used for each food concentration for each strain. A second series was repeated at the critical points. The results are given in table II.

TABLE II

EFFECT OF QUANTITY OF FOOD UPON TYPE OF SPORANGIA

Potato decoction

Per cent concentration	Strain A		Strain X	
	Per cent sporangia	Per cent sporangiola	Per cent sporangia	Per cent sporangiola
0.0	50	50	100	0
0.1–1.0	50	50	100	0
1.0–13.0	50	50	100	0
14.0	50	50	90	10
20.	50	50	100	0
40.	40	60	85	15
60.	30	70	70	30
90.	30	70	70	30

Potato-dextrose solution

0.5	50	50	100	0
1–3	40	60	100	0
5–7	40	60	85	15
10	30	70	85	15
20–100	30	70	70	30

On both media Strain X produced only sporangia with poor food supply. Strain A never produced sporangia alone, although in media with low food supply there were relatively more sporangia and fewer sporangiola. The sporangia produced by both strains with low food concentrations were much smaller than those produced with greater food supply. Most of the sporangia produced upon potato agar between concentrations of 0.5 and 5 per cent were 10–16 μ in diameter and contained from 10 to 20 spores (pl. 25, fig. 7). Sporangia produced between 10 and 100 per cent concentrations were largely 40–80 μ in diameter and contained 50–100 spores (pl. 25, fig. 10). Upon the potato-dextrose agar between concentrations of 0.5 and 10 per cent the sporangia were 10–16 μ in diameter with 10–20 spores. Above 10 per cent they were 40–80 μ in diameter and contained 50–100 spores.

The concentration of the food also had its effect upon the sporangiola.[1] Between concentrations 0.5 and 5 per cent of potato agar and 0.5 and 2 per cent of potato-dextrose agar there was a predominance of solitary sporangioliferous heads. There were also sporangiola borne upon 2 sporangioliferous heads and a few upon 3 and 4 heads (pl. 25, figs. 1–4). As the amount of food increased, the sporangioliferous heads increased in number until there were as many as 20–30 in the higher concentrations of the two kinds of media (see pl. 25, fig. 5).

As the concentration of food increased the mycelium became more copious, particularly in the case of Strain X. After 70 hours this strain developed a dense mycelium covering the whole petri dish. In the aerial mycelium sporangia 10–16 μ in diameter began to develop and were very numerous in 4 days. This same type of sporangia appeared with Strain A but de-

[1] Cunningham states concerning the conidial fructifications of *Choanephora Cunninghamiana:* "In cases in which nutrition is imperfect, only a small number of capitella are produced and filaments are encountered with numbers diminished through various degrees until we find specimens with only two capitella. The process of abortion doesn't, however, reach its climax here; for a further stage occurs in which no capitella are produced, and in which the dilated extremity of filament gives direct origin to the sterigmata."

velopment was much slower, from 6 days to 2 weeks being required for a rich growth of aerial sporangia to develop.

Cultures of potato-dextrose agar with good mycelial development were autoclaved, cooled, and reinoculated. After 48 hours, fruiting appeared. In Strain A an equal number of both types of sporangia were produced. Most of the sporangiola were borne upon the solitary sporangioliferous heads (pl. 25, fig. 1). The sporangia were of the small size. Strain X produced no sporangiola and only small sporangia (pl. 25, fig. 7). Mycelium was scant in both strains. Apparently no toxic products of metabolism which inhibit growth were produced, since the results were practically the same as in the small concentrations of nutrient. Warm agar was poured over 6-day-old colonies of Strain A and Strain X grown upon potato-dextrose agar. In 48 hours fruiting appeared in both strains. The results were the same as in the killed cultures. The experiment was repeated with 1, 2 and 5 per cent potato agar with practically the same results. On media with a minimum of food supply, Strain A produced both types of sporangia while Strain X produced one type only.

A final experiment was tried to test the effect of poor nourishment upon the distribution of sporangial types. A piece of mycelium, 1 mm. in length and 3 months old, was transferred to a petri dish containing agar. Other pieces of similar mycelia were transferred to potato-dextrose agar. Fruiting appeared in 48 hours upon the potato dextrose and in 72 hours upon the agar.

TABLE III

EFFECT OF NOURISHMENT UPON TYPE OF SPORANGIA

Medium	Strain A		Strain X	
	Per cent sporangia	Per cent sporangiola	Per cent sporangia	Per cent sporangiola
Potato dextrose (10 dishes)	35	65	85	15
5 cc. potato broth (10 dishes)	50	50	100	0
Agar (10 dishes)	95	5	100	0

The above table shows the importance of nourishment and its effect upon the type of sporangia produced. Thus in Strain A, it was possible to obtain almost 100 per cent sporangia with very poor nourishment. A single spore culture upon agar-agar produced the same distribution of sporangial types as the piece of mycelium.

EFFECT OF HUMIDITY AND OF MOISTURE IN SUBSTRATE

On potato-dextrose agar the fungus produced a predominance of sporangiola about the edge of the petri dishes. This suggested that a dry substrate might help in the production of sporangiola. The dryness was obtained experimentally by varying the amounts of agar.

Strain A was planted on potato-dextrose agar, the concentration of agar in the media being 0.7, 0.9, 1, 1.2, 1.5, 2, 3, 4, 5, 6 per cent. As the concentration increased, the mycelium became heavier and more matted. The distribution of sporangial types was not affected between agar concentrations 0.7 and 3 per cent. The media containing 5 and 6 per cent agar had very much mycelium, but there was a little less than the usual quantity of sporangiola about the edge of the dish. There were numerous sporangia of the smaller dimension (10–16 μ) in the aerial mycelium on the petri dishes containing the higher concentrations of agar (5–6 per cent). These experiments were repeated, using two petri dishes for each concentration of agar. The same procedure was followed with Strain X, and here also the growth of mycelium was greater upon the media containing 2 to 6 per cent agar. In the 3, 4, 5, and 6 per cent agar concentrations the mycelium was unusually dense, with a preponderance of sporangia almost entirely in the aerial portion. Only a few sporangiola (4–5 per petri dish) were found in these higher agar concentrations.

The above experiments were repeated with the same series of concentrations of agar in potato decoction. The potato decoction was diluted (1 and 2 cc. solution to 100 cc. distilled water) and made up with the same series of agar concentrations. The results for both strains were the same as in potato-dextrose agar. The distribution of sporangial types remained the same

on the dry media as on the moist—for Strain A about 60 per cent sporangiola to 40 per cent sporangia and for Strain X only sporangia. It was noticed upon the drier substrates that the sporangia and sporangiola were borne upon sporangiophores about 5 mm. longer than the normal ones.

Finally a dilute potato broth (5 cc. potato decoction in 100 cc. distilled water) with the same agar concentrations was used. The distribution of sporangia and sporangiola was not affected, Strain A having 50 per cent of each and Strain X only sporangia. Both strains were then grown upon liquid potato decoction, and fruiting appeared in two days in both. Strain X in 20 tubes produced all sporangia, while Strain A produced 70 per cent sporangiola and 30 per cent sporangia.

The two strains were then grown in different humidities, the procedure of N. Stevens ('16) being followed. Large jars which could be sealed were used as containers. The petri dishes with lids removed were placed in the jars and the jars incubated at 20° C. The media used were potato-dextrose agar and potato agar (5 cc. potato decoction to 100 cc. water). Both strains were grown in the following humidities: 100, 90, 80, 70, 60, 42, 21.5, 10.5 and 2.5 per cent. The distribution of sporangial types was not affected in either strain grown in the range of humidity between 100 and 21.5 per cent. At 10.5 and 2.5 per cent, Strain A showed a slight increase in the number of sporangiola, but this increase was too slight to be of real significance; Strain X was not affected. The strains were also grown in very moist agar in the jar at 100 per cent humidity, without effect upon the distribution; nor was there any difference in distribution when the two strains were grown upon 7 per cent potato agar at 2.5 per cent humidity.

These results in general agree with Bachmann's ('95) prediction that humidity has very little effect upon the production of different sporangial types. However, in growth upon liquid potato broth, sporangia only were produced in Strain X. When this broth was solidified by the addition of agar, the usual distribution occurred, 90 per cent sporangia, 10 per cent sporangiola. Strain A did not behave in this manner.

EFFECT OF BASIC FUCHSIN

Since growth was rather much restricted for both strains upon Endo's agar, it was thought that the basic fuchsin present in the media might be the cause. In order to see whether such an inhibitor of growth would influence the distribution of sporangial types potato-dextrose agar and potato agar were prepared containing varying amounts of basic fuchsin.

The potato broth was made up by mixing 5 cc. of potato decoction with 100 cc. of water and solidifying with 2 gms. of agar. Basic fuchsin was then added in amounts ranging from .0001 to .01 per cent. At .01 per cent basic fuchsin there was no growth, but as the amount was decreased mycelial growth became more abundant. Fructification did not appear until 72 hours after inoculation. Many of the sporangia and sporangiola did not mature during the whole life of the colony, that is, they remained white throughout their life period. This was especially true when the potato agar contained .005, .004, and .003 per cent basic fuchsin. As far as distribution of sporangial types was concerned, there was only a slight increase in the number of sporangiola in Strain A where the higher concentrations of basic fuchsin were used. With Strain X, the distribution did not seem to be influenced at all, for 100 per cent sporangia was produced, the usual type of fructification when 5 cc. potato solution is used. This experiment was repeated, and two petri dishes were used for each basic fuchsin concentration for both strains. Potato dextrose was next used, the basic fuchsin varying from .0001 to .025 per cent concentration. Strain X did not grow when the dye concentration was .025 per cent, but with the lower concentration the phenomena mentioned above were also observed. There was the same slight increase in sporangiola number for both strains. Mycelial growth was also restricted and the fructifications took much longer to mature, some never maturing. In conclusion, it may be stated that making conditions unfavorable for growth and fruiting by introducing a dye, basic fuchsin, into the substrate does not seem to have any marked effect upon the distribution of sporangial types in either strain.

EFFECT OF INCUBATION TEMPERATURE

The two strains were inoculated upon potato-dextrose agar and incubated at different temperatures. At 6° C. there was no growth, but at 22° C. normal growth began and the usual types of sporangia resulted in both strains. Low temperature merely suspends activity. The optimum temperature was between 18° C. and 22° C. Between 28° and 36° C., the distribution of sporangial types was not affected but there was a slight increase in mycelium. At 34–36° C. there was very little fructification. When the fungi were incubated at 37° C. only mycelium was produced and at 40° C. there was no growth. The temperature at which strains are incubated seems to have no effect upon the distribution of sporangial types.

EFFECT OF EXPOSURE OF SPORES TO HIGH TEMPERATURES

The procedure followed in this experiment was similar to that of Barnes ('35) who worked with *Thamnidium elegans*. With a pipette, 0.5 cc. of a spore suspension of both strains was transferred to many tubes. These tubes were exposed to various high temperatures for different time intervals. Two petri dishes were inoculated for each high-temperature exposure for both strains. The whole experiment was performed twice.

The following are temperatures and time intervals at which the strains were exposed:

45° C.—1 min., 2 min., 5 min., 10 min., 1 hour, 2 hours.
50° C.—1 min., 2 min., 5 min., 10 min., 1 hour, 2 hours.
55° C.—1 min., 2 min., 5 min., 10 min.
60° C.—1 min., 2 min., 5 min., 10 min., 20 min., 30 min.
65° C.—1 min., 2 min., 5 min., 10 min., 15 min., 20 min.
70° C.—1 min., 2 min., 5 min., 10 min., 15 min., 30 min.
75° C.—1 min., 2 min., 5 min., 10 min., 15 min., 30 min.
80° C.—1 min., 2 min.
90° C.—15 sec.
100° C.—5 sec., 15 sec.

The spores were killed at 80° C. when exposed for one minute. The distribution of sporangial types was not at all affected in either strain by an exposure to high temperature. The only noticeable effect was a retardation in growth and the appear-

ance of fructification at 72 hours instead of the customary 48 hours when the spores were heated at 55° C. and above. Barnes ('35) found that spores of *Thamnidium elegans* yielded variant cultures after exposure to moderately high temperatures, and that the variants preserved their distinguishing characters through a considerable number of transfers. She does not mention whether the high temperature influenced the distribution of sporangia and sporangiola.

Frozen spores were thawed out and inoculated upon potato dextrose and incubated at the optimum temperature. Freezing apparently had no effect upon the distribution of sporangial types.

EFFECT OF LIGHT

The two strains were incubated in total darkness and in the light produced by a 60-watt lamp. The distribution of sporangial types was not affected. Light seems to be a stimulus for mycelial growth because those petri dishes in the light always produced much more mycelium than those in the dark.

EFFECT OF HYDROGEN-ION CONCENTRATION

Both strains grew best upon slightly acid media. The potato-dextrose agar has a pH of 5.4, and potato agar varied between 5.4 and 5.7. Other media used had various hydrogen-ion concentrations: bacto-malt-extract agar 4.6, potato-dextrose agar 5.4, nutrient caseinate agar 6.5, nutrient agar 6.6, eosine methylene-blue agar and yeast-dextrose agar 7.0, Endo's agar 7.52, bacto brain-veal agar 7.6. The distribution of sporangial types was not affected except on Endo's agar and peptone agar where other factors than hydrogen-ion concentration probably produced the results.

Potato dextrose was then made up with varying amounts of molar potassium hydroxide, and the pH measured by the glass electrode method. The hydrogen-ion concentrations studied were 5.6, 6.2, 7.3, 8.7, 9.6 and 10.2. The distribution of sporangial types was not at all affected by the hydrogen-ion concentration. Neither strain grew upon the media which has a pH of 10.2. At pH 9.6 growth was somewhat restricted, that is, there was less mycelium, but the distribution of sporangia

or sporangiola was not markedly changed. At a pH of 9.6 in Strain X there was a slight increase in sporangiola. Instead of the usual distribution, which is about 70 per cent sporangia and 30 per cent sporangiola, there was about an equal number of both types of fruiting bodies. It should be remembered that Endo's agar has a pH of 7.52, which is slightly on the basic side. Upon that agar there were more sporangiola than upon potato-dextrose agar. The slightly alkaline pH might play a slight role. A medium definitely upon the acid side, as bacto-malt-extract agar with a pH of 4.6, has no effect upon the distribution of sporangial types.

SUMMARY

1. Two strains of *Blakeslea trispora* reacted differently to various environmental stimuli. At times, they were so different in growth reactions that it was a temptation to separate the two strains as distinct species.

2. The kind of medium did not seem to have any marked effect upon the distribution of sporangial types, except Endo's agar and peptone agar with Strain A. The formation of sporangiola was favored by these media.

3. The quantity of the food has the most direct influence upon the distribution of sporangial types. With poor nourishment Strain X produced only sporangia. With richer food or with addition of various sugars to potato agar, the sporangiola began to appear. With Strain A it was never possible to obtain one type of reproductive structure to the exclusion of the other —even when agar alone was used. Here the distribution of reproductive structures was half sporangia and half sporangiola. When a tiny piece of mycelium was planted upon agar, there was 95 per cent sporangia to 5 per cent sporangiola. In general, media poor in nutrients favor sporangial formation while those rich in nutrients favor sporangiole formation.

The various other stimuli tried—humidity, moisture content of the substrate, light, hydrogen-ion concentration, freezing and heating of spores, prolonged soaking of spores in distilled water, various methods of inoculation, various incubation temperatures, and frequent transplantation—had no effect upon the distribution of sporangial types. The extent of mycelial

growth and pigmentation were affected by some of these stimuli. In general, the most important factor which determines the type of sporangial fructification is the quantity of food, all other environmental factors mentioned playing a relatively minor role.

This work was done at the suggestion and under the supervision of Professor Carroll W. Dodge in the Henry Shaw School of Botany of Washington University. The writer is greatly indebted to Professor Dodge for many helpful suggestions during the course of the experiments. Also, it is a pleasant duty to acknowledge the help in experimental procedures rendered him by his classmate, Alexander Horwitz.

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EXPLANATION OF PLATE

PLATE 25

Blakeslea trispora Thaxter.

Figs. 1–4. Sporangioliferous heads produced under conditions of poor nourishment. × 140.

Fig. 5. Fructification produced under conditions of abundant nourishment. × 140.

Fig. 6. Spore showing cluster of delicate radiating appendages. × 900.

Fig. 7. Smaller type of sporangium with no columella. × 350.

Fig. 8. Typical sporangiolum with three spores showing small spherical vesicle which attaches the sporangiolum to the sporangioliferous head. × 490.

Fig. 9. Sporangioliferous head with adhering sporangiola. × 250.

Fig. 10. Larger type of sporangium with columella. × 250.

Fig. 11. Type of sporangium figured by Thaxter but never observed by the writer, which greatly resembles the sporangiolar type of fructification. × 240.